

# The Occurrence of *Thiobacillus ferrooxidans* and Arsenic in Subarctic Streams Affected by Gold-Mine Drainage

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**ABSTRACT.** Thirty-five streams in gold-mining regions between Rampart, Alaska, and Dawson City, Yukon Territory, were sampled to determine dissolved arsenic concentrations, and numbers of the acidophilic iron- and sulfur-oxidizing bacterium *Thiobacillus ferrooxidans*. The pH of the streams varied from 6.3 to 8.6 and the streams were nearly saturated with dissolved oxygen. *T. ferrooxidans* was found in eight of nine streams affected by gold-mine drainage and in only one of 26 streams not affected by gold-mine drainage. Some of the streams affected by gold-mine drainage near Fairbanks, Alaska, occasionally contained levels of dissolved arsenic above 50 parts per billion. The recognition that *T. ferrooxidans* is associated with gold-mine material and that the heavy metal arsenic exists in streams affected by gold-mine wastes is important for understanding the environmental effects of mining activity on subarctic streams.

**Key words:** arsenic, gold mines, heavy metals, pyrite, *Thiobacillus ferrooxidans*

**RÉSUMÉ.** Trente-cinq ruisseaux dans les régions minières d'or entre Rampart, en Alaska, et Dawson, au Yukon, ont été échantillonnés afin de déterminer les concentrations d'arsenic qui y étaient dissoutes, ainsi que le nombre de bactéries *Thiobacillus ferrooxidans* acidophiles oxidant le fer et le soufre. Le niveau des pH des ruisseaux variait entre 6.3 et 8.6 et les ruisseaux étaient presque saturés d'oxygène dissous. Des traces de *T. ferrooxidans* ont été relevées dans huit des neuf ruisseaux touchés par le réseau de drainage des mines d'or, tandis que seulement un des 26 ruisseaux non touchés par le réseau en présentait des traces. Certains des ruisseaux touchés par le réseau de drainage des mines d'or près de Fairbanks, en Alaska, contenaient à l'occasion des niveaux d'arsenic dissous de plus de 50 parties par milliard. La reconnaissance de l'association de *T. ferrooxidans* aux produits de mines d'or et de la présence d'arsenic de métal lourd dans les ruisseaux contenant des déchets de mines d'or contribue de façon importante à la compréhension des effets de l'activité minière sur les ruisseaux sub-arctiques.

**Mots clés:** arsenic, mines d'or, métaux lourds, pyrite, *Thiobacillus ferrooxidans*

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## INTRODUCTION

Arsenic is enriched in gold and sulfide deposits throughout many areas of Alaska and Canada. Consequently, some stream waters, stream sediments and groundwaters contain dissolved arsenic concentrations in excess of the Environmental Protection Agency's recommended drinking water standard limit of 50 parts per billion (ppb; Wilson and Hawkins, 1978). Microorganisms of the genus *Thiobacillus* catalyze many of the thermodynamically feasible but kinetically slow reactions that lead to dissolution of heavy metals from naturally occurring minerals. The acidophile *Thiobacillus ferrooxidans*, in particular, is capable of oxidizing reduced iron and many reduced sulfur compounds including arsenopyrite (Ehrlich, 1964). *T. ferrooxidans* is normally found in acid streams draining from mines that have pyrite deposits, but these bacteria have also recently been found in neutral and alkaline waters affected by coal strip-mining in southeastern Montana (Olson *et al.*, 1979). Since placer, suction dredge and lode gold-mining have recently increased dramatically in Alaska, many streams are now periodically perturbed with suspended material from mine wastes (Zemansky *et al.*, 1976). As part of a study concerning the biogeochemistry of arsenic, we have analyzed a variety of streams affected by gold-mine drainage in subarctic Alaska and Canada to determine the concentrations of dissolved arsenic and numbers of *T. ferrooxidans* present. We undertook this portion of the study because we suspected that if high numbers of *T. ferrooxidans* are associated with suspended material from gold-mine drainage, heavy metals (e.g., arsenic) could be leached

from this material (see Dugan, 1972). We report here that the acidophilic microorganisms are present in most neutral-pH subarctic streams affected by gold mining. Some of these streams have measurable levels of dissolved arsenic (>5 ppb), others do not.

## MATERIALS AND METHODS

### Sampling Sites

Five stations on four streams near Fairbanks, Alaska, were periodically sampled throughout 1980 and 1981. Several placer mines in the Tanana River watershed affect some of these streams. The sampling site in Eva Creek was a few hundred yards below a placer operation which has a heavy impact on this small stream. Goldstream Creek is a larger stream indirectly affected by mine drainage from a few smaller tributaries. There were two sampling sites on Goldstream Creek. Site 1 was several stream miles from the nearest active mine and site 2 was approximately five stream miles downstream from site 1 with no active mining between the sites. Mine drainage currently has no direct impact on Ester Creek, but the creek flows through several acres of placer mine tailings. Engineer Creek is also not directly affected by mine drainage, but known pyritic deposits occur along its bed (Wilson and Hawkins, 1978). Several remote sampling sites throughout interior Alaska and Yukon Territory, Canada, were sampled during the summer of 1981. Evidence of mining activity in remote sampling areas was indicated by visual or measured stream turbidity and occasionally by personal sightings of mines.

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TABLE 1. Some biogeochemical characteristics of streams in the Fairbanks mining district, 1980

Stream	Water Temperature	pH	<i>Thiobacillus ferrooxidans</i>	<sup>1</sup> Arsenic (not fixed)	<sup>1</sup> Iron (not fixed)	Turbidity
	°C		<sup>2</sup> MPN/ml	PPB	PPM	
Eva	8	7.0	35	222	104	3000
	9	6.5	35	13	17	15
	6	6.4	160	16	6	21
	5	6.3	35	0	1	33
	5	—	110	347	557	3500
Ester	11	8.0	240	25	2	14
	10	7.6	240	20	1	4
	12	7.2	>2400	12	76	600
	0	8.3	35	9	4	3
	-1	7.8	92	—	—	—
Goldstream	9	7.8	1.1	31	17	150
	10	—	4.9	70	56	625
	8	7.7	2.2	34	17	—
	0	—	31	19	—	190
Engineer	8	7.2	0.4	—	3	5
	8	7.6	2.7	26	1	6
	8	7.3	0.5	49	3	8
	11	7.4	2.2	58	5	18
	8	7.7	1.3	40	2	—
	6	7.6	0.5	30	2	6
	3	7.4	4.9	11	—	6

<sup>1</sup>Samples were acidified to pH ≈2 and then filtered.

<sup>2</sup>Most probable number per ml.

<sup>3</sup>Nephelometric Turbidity Units.

#### Sampling Methods

Grab samples were taken by wading into each stream and filling sterile plastic bottles for microbiological analysis; some plastic bottles were rinsed with nitric acid and contained nitric acid for total and dissolved arsenic, and some plastic bottles were rinsed in hydrochloric acid and contained hydrochloric acid for total and dissolved iron (American Public Health Association, 1975). Some samples were filtered at the time of collection through 0.45 micrometer pore-size membrane filters before acidification for dissolved arsenic determinations, while other samples were filtered after acidification for acid mobile (not fixed) iron and arsenic determinations.

At the time of sampling, the water temperature, pH, dissolved oxygen and turbidity were measured with a Horiba Model U-7 field water-sampling kit. The grab samples were immediately placed in an ice chest. The samples used for iron and arsenic measurements were either frozen upon return to the laboratory or analyzed within two hours of collection. The samples used for *T. ferrooxidans* enumeration were inoculated into most probable number (MPN) tubes within a few hours of collection either in the laboratory, or in the field when necessary.

#### Microbiological Methods

The culture medium used for standard MPN determination of *T. ferrooxidans* was modified from Tuovinen and Kelly (1974) and contained: FeSO<sub>4</sub>·7H<sub>2</sub>O (30 g/l), MgSO<sub>4</sub>·7H<sub>2</sub>O

(0.4 g/l), (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (0.4 g/l), KH<sub>2</sub>PO<sub>4</sub> (0.1 g/l), and 10N H<sub>2</sub>SO<sub>4</sub> to bring the medium to pH 1.8 - 2.0. The medium was clear and remained so indefinitely. If, after a sample (or diluted sample) was inoculated into a test tube containing this medium and incubated, a dark reddish-brown color and/or precipitate appeared, the sample (or diluted sample) was considered positive for the presence of *T. ferrooxidans*. The color change usually occurred within three weeks, but MPN tubes were retained for at least six weeks before taking final readings.

#### Chemical Methods

Total-arsenic and dissolved-arsenic analyses were performed using the atomic absorption method described by Wilson and Hawkins (1978) as modified with a nickel matrix (Ediger, 1975). Arsenate ion was determined in some of the samples by treating the samples with a modified Murphy and Riley (1962) molybdate reagent, extracting the arsenate with isoamyl alcohol, analyzing the aqueous phase by atomic absorption (Brown and Button, 1979) and subtracting the arsenic concentration in the aqueous phase from the total arsenic concentration in the samples. Acid mobile (not fixed) iron was determined by atomic absorption spectrophotometry.

#### RESULTS AND DISCUSSION

Tables 1 and 2 show the MPN of *Thiobacillus ferrooxidans* in relation to dissolved arsenic and iron concentrations,

TABLE 2. Some biogeochemical characteristics of streams in subarctic Alaska and Canada, 1981

Date	Stream	<sup>5</sup> Mining Activity	Water	pH	<i>Thiobacillus ferrooxidans</i>	<sup>1</sup> Total	Dissolved	<sup>3,5</sup> Turbidity
			Temperature			Dissolved As	O <sub>2</sub>	
			°C		<sup>2</sup> MPN/ml	ppb	ppb	ppm
06/05/81	Goldstream 1	p	7.5	8.1	0.5	59	10.2	34
06/05/81	Goldstream 2	p	—	—	<sup>4</sup> ND	—	—	—
07/10/81	Goldstream 1	p	6.5	7.7	2.3	7	11.6	179
07/10/81	Goldstream 2	p	6.9	7.8	2.3	< 5	10.6	155
08/10/81	Goldstream 1	p	9.2	8.4	0.4	8	11.8	153
08/10/81	Goldstream 2	p	10.3	8.0	0.2	< 5	11.6	175
06/25/81	Hess	a	15.0	7.0	ND	< 5	—	—
06/25/81	Mile 41	a	12.0	7.0	ND	< 5	—	—
06/25/81	Little Doll	a	19.9	7.2	ND	< 5	8.8	15
06/26/81	Ray River	a	16.8	7.9	ND	< 5	9.5	12
06/26/81	S. Bonanza	a	6.0	6.8	ND	< 5	—	—
06/26/81	Fish	a	7.5	6.9	ND	< 5	—	—
06/27/81	Livengood	p	6.7	6.9	1.3	< 5	11.7	Above limit
06/27/81	Tatalina	p	4.8	7.3	2.3	< 5	13.0	278
06/27/81	Tolovana	a	12.5	7.7	ND	< 5	9.6	3
08/03/81	Woodchopper	a	9.0	6.1	ND	< 5	10.2	a
08/04/81	Coal	p	8.1	8.0	1.3	< 5	11.3	a
08/04/81	Charlie	a	15.3	7.7	ND	< 5	10.1	a
08/04/81	Kandik	a	10.0	7.7	ND	< 5	10.7	a
08/04/81	Nation	a	9.6	7.9	ND	< 5	10.9	a
08/04/81	Tatonduck	a	8.4	7.6	ND	8	—	a
08/06/81	Klondike	a	12.1	7.7	ND	< 5	11.1	p
08/06/81	Thaine	a	11.3	7.9	ND	< 5	11.2	a
08/06/81	Fresno	a	7.0	7.9	ND	< 5	15.0	a
08/06/81	Fifteen Mile	a	10.8	8.1	ND	< 5	11.7	a
08/06/81	Cassiar	a	8.1	8.5	ND	< 5	13.9	a
08/07/81	Fortymile	a	13.0	7.4	ND	< 5	10.2	a
08/07/81	Eagle	a	8.3	8.3	ND	< 5	13.0	a
08/07/81	American	a	10.1	8.2	ND	< 5	11.6	a
08/07/81	Seventymile	p	12.1	7.7	0.2	< 5	12.5	p
08/07/81	Sam	a	9.4	7.2	ND	< 5	11.8	a
08/08/81	Thanksgiving	a	9.0	7.7	ND	8	12.7	a
08/08/81	Lower Birch	p	15.6	7.9	ND	6	11.7	p
08/08/81	Crooked	p	15.4	7.8	0.2	< 5	14.1	p
08/08/81	Ketchum	a	9.5	8.1	ND	< 5	13.5	a
08/08/81	Bedrock	a	9.8	8.6	ND	< 5	15.3	a
08/08/81	Circle Hot Springs	a	48.7	7.8	ND	< 5	5.0	a

<sup>1</sup>Samples were filtered and then acidified to pH ≈ 2.

<sup>2</sup>Most probable numbers per ml.

<sup>3</sup>Only visual determinations were made after 81/06/27.

<sup>4</sup>ND = No *Thiobacillus ferrooxidans* detected.

<sup>5</sup>p = present.

a = absent.

temperature, pH and presence of mining activity in all of the streams sampled. The pH varied from 6.3 to 8.6 and oxygen was nearly saturating in every stream sampled. The highest MPNs for *T. ferrooxidans* occurred at sampling sites nearest mining disturbances. For example, the sampling site on Eva Creek (which always had the highest MPN) was 200 yards from an active placer mine. When sites 1 and 2 on Goldstream Creek were sampled on the same day, site 1 (which was closer to mining activity than

was site 2) never had a lower MPN than did site 2 (Table 2). Table 2 also shows the variability of MPN with pH, arsenic concentration and temperature. Since none of the streams sampled were glacier-fed, turbidity was a good indicator of mining activity *per se*. However, turbidity was not a good indicator of the type of mining activity that was occurring at the time of sampling (sluicing, overburden removal, shutdown, etc.). For this reason no direct correlations are apparent between turbidity and any of the

measured parameters. For example, Table 1 shows that the arsenic content of Eva Creek was highest when most turbid, while the arsenic content of Ester and Goldstream creeks was relatively constant. Similarly, the MPN of *T. ferrooxidans* was consistently highest in Ester Creek which was normally less turbid than the other creeks that we periodically sampled (Table 1). However, except for Engineer Creek which is exposed to pyritic deposits, we could not detect any *T. ferrooxidans* in streams which were not affected by mine drainage (Table 2).

These results suggest that *T. ferrooxidans* is not growing in the streams, but large populations are merely being washed down with particulates which may also contain heavy metals (e.g. arsenic). This interpretation was also postulated by Olson *et al.* (1979) who isolated *T. ferrooxidans* in neutral pH streams in eastern Montana. Thus, the source of the bacteria is probably pyritic material associated with the gold deposits. Ross and Ivarson (1981) report the occurrence of *T. ferrooxidans* in pyrite-containing shales as far north as Ellef Ringnes Island in arctic Canada. Large pyrite deposits have not been reported near most placer gold deposits. Thus pyrite (if present) must occur only in localized areas within the material removed or sluiced during gold mining (see Chadwick *et al.*, 1975). Such localized zones are visibly evident throughout Canada (Ross and Ivarson, 1981), below the surface in a lode mine in Ester Dome, Alaska (Luong *et al.*, 1981), and pyrite does appear in gold miners' sluice boxes in many of the streams we sampled.

Arsenic is commonly found to be associated with gold deposits in Alaska (Wilson and Hawkins, 1978) and Canada (Smecht *et al.*, 1975; Boyle, 1979). Dissolved arsenic in amounts >50 ppb was present only in streams near Fairbanks, Alaska, whether gold mining was occurring or not (Tables 1 and 2). Thus, while *T. ferrooxidans* was detected in all but one stream affected by gold mining, high levels of arsenic in streams and mining activity on those streams are not directly related (Tables 1 and 2). On the other hand, clear groundwater from the previously mentioned gold and silver lode mine in Ester Dome, Alaska, contains nearly 500 ppb dissolved arsenic and yet we have never detected *T. ferrooxidans* in groundwater samples. We believe that this is simply a further indication that the microbial population is localized and associated with particulates which are washed downstream with mine wastes. If the particulates contain heavy metals associated with sulfides, the resident microbial flora may have contributed to the release of the heavy metals from the material. This is supported by studies that have shown that *T. ferrooxidans* can catalyze the release of arsenic from pure arsenopyrite (Ehrlich, 1964) and gold-mining material collected from Ester Dome (Luong *et al.*, 1981); however, the exact mechanism of action is not known. The arsenic may be released by direct oxidation of the material, or the material may be oxidized by ferric ions that have been produced by microbial oxidation of ferrous ions in surrounding waters. Once the

arsenic is released, it should remain stable as a soluble arsenite ( $\text{As}^{3+}$ ) ion under slightly reducing conditions, but should spontaneously oxidize to arsenate ( $\text{As}^{5+}$ ) under oxidizing conditions. Figure 1 shows chemical speciations of arsenic for three sample sites on two occasions. Greater than 50% of the total dissolved arsenic (filtered and immediately fractionated) can occur in forms less oxidized than  $\text{As}^{5+}$  in these sediment-laden (Eva) and clear (Engineer and Ester) creeks.

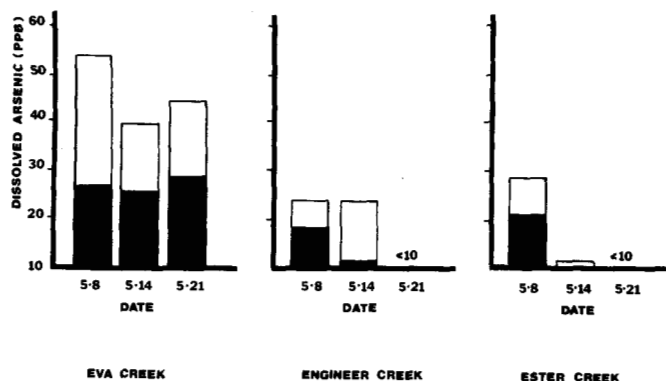


FIG. 1. Speciation of dissolved arsenic in three streams on three sampling dates in 1981. Shading indicates the portion of the total dissolved arsenic that was not extracted by the molybdate reagent. The unshaded bar represents the arsenate (molybdate extractable) portion of the total dissolved arsenic present.

The fact that  $\text{As}^{5+}$  is not the only dissolved form of arsenic in solution at neutral pH under oxidizing conditions (Fig. 1) suggests that abiotic spontaneous oxidation of arsenic is kinetically limited (Hounslow, 1980). It is theoretically possible that oxidation of  $\text{As}^{3+}$  ions could also be catalyzed by *T. ferrooxidans*, since other reduced metals are oxidized by this microorganism (Tuovinen and Kelly, 1974). As  $\text{As}^{5+}$  is formed (either chemically or biologically), it should largely be removed from the dissolved phase by precipitation with ferric hydroxides to produce the mineral scorodite (Wilson and Hawkins, 1978).

#### CONCLUSIONS

In summary, we have found that high numbers of acidophilic bacteria exist in all but one stream directly or indirectly affected by gold mining. Some of these streams contain high levels of arsenic. Since previous studies have suggested that the very presence of *T. ferrooxidans* in mine water indicates that these organisms have already oxidized pyrite (Dugan, 1972), their presence in the streams sampled in this study could indicate that arsenic (from arsenopyrite) or other heavy metals (from other reduced sulfides) have been released. Neutral-pH aerobic waters with high sediment loads (streams affected by mine wastes) should eventually immobilize dissolved arsenic by precipitating or complexing with ferric hydroxides. The conditions regulating the rate of precipitation and/or sorption in each stream are, of course, variable with time and type

of perturbation (Hounslow, 1980). Until more is known about the biogeochemical processes attendant to gold mining in subarctic streams, environmental impacts to streams and groundwaters affected by mining will be difficult to assess.

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